Water Binding in Whey Protein Concentrates

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Abstract

Measurements of the heat of fusion of free water in concentrated solutions of purified whey proteins showed that .5 g water/g whey protein would not freeze at −40 C. This water was defined as bound. Total bound water in protein solutions containing lactose and salts varied between .5 and 1.2 g water/g solids, with unfreezable water increasing as the concentrations of lactose and salts were increased. Bound water values observed with several whey protein products agreed with values computed from data both for high and low molecular weight fractions of these products. Thermal denaturation did not cause significant changes in water binding. Water vapor sorption by a concentrated whey protein product was an additive process at all relative pressures, with the amount of sorbed water, as P/P_0 approached unity, equal to the unfreezable water in the corresponding suspension of the same product.

Introduction

The suspended and dissolved materials in liquid cheese whey bind much water making whey difficult and expensive to concentrate or dry, thus magnifying the cheese industry's whey disposal problems. Study of water binding by whey solids should aid in the solution of these problems. This study determined the significance of the protein fraction, its state of denaturation, and the contribution of the low molecular weight fraction to water binding in whey products, especially the whey protein concentrates currently under development (13).

In this paper, bound water is defined as that which remains unfrozen at low temperatures (< −40 C). Actually, Kuprianoff (11) has qualified this definition by recommending that unfreezable water be called water bound against freezing. We determined such bound water calorimetrically in concentrated aqueous dispersions of several whey protein products and fractions thereof. These data describe water binding at high water activity and were supplemented with water vapor sorption measurements with dried samples to determine how water vapor uptake is influenced by composition at all relative pressures.

Materials and Methods[1]

Spray-dried whey protein concentrates were selected to provide a wide range in protein content (Table 1). The products were either manufactured in commercial plants or prepared in the Dairy Products Laboratory Pilot Plant. Protein concentration was by methods utilizing either ultrafiltration, electrodialysis, gel permeation, or a combination of these techniques. The detailed procedures used in the commercial plants, however, have not been fully disclosed to us.

Protein concentrates containing 35, 55, and 78% protein were separated into high and low molecular weight fractions by suspending the powders in distilled water and dialyzing the suspensions against repeated changes of distilled water. The dialyzable and nondialyzable fractions were recovered and freeze-dried for further study.

Purified whey protein was prepared by precipitating casein from skim milk with HCl, filtering, and exhaustively dialyzing the fluid whey against distilled water to remove lactose and salts.

Unfreezable water was determined as described (5) with the Perkin-Elmer Model DSC-1B differential scanning calorimeter. Measurements were with small samples (3 to 7 mg) of concentrated aqueous dispersions, containing 65 to 75% H_2O, prepared from the dried powders.

Pellets of insoluble protein were isolated by suspending powder samples in water and centrifuging at 78,000 × g for 20 min in the 30

TABLE 1. Unfreezable water content and chemical composition of the whey protein concentrates.

Material	Protein	Lactose	Ash	Denatured protein	grams unfrozen H_2O / gram solids
	(% of dry weight)			(% of total protein)	
Commercial products					
A	35.0	55.0	3.0	41.0	.91
B	55.0	25.6	10.0	48.6	1.09
C	73.5	13.3	4.1	18.5	.51
D	72.8	7.2	13.8	14.3	.50
E	87.0	4.0	1.6	17.6	.47
Pilot plant products					
F (Empro-80)	78.0	1.3	12.8	29.9	.54
G	83.0	5.5	5.3	62.5	.46
H	87.1	6.6	1.3	15.4	.48

rotor of the Spinco Model L preparative ultracentrifuge. Sections of the pellets of insoluble protein then were examined calorimetrically for unfreezable water content.

The possible relationship between protein denaturation and bound water also was studied directly in the calorimeter. After measuring the amount of freezable water, the protein sample, sealed in an aluminum capsule, was heated rapidly (80°/min) to 85 C in situ in the calorimeter cell and held there for 1 h to complete denaturation (9). The sample then was cooled to redetermine freezable water and assess effects of denaturation on water binding.

Water vapor sorption was determined gravimetrically with the Cahn RG recording electrobalance installed in a custom-made glass adsorption system (2). Sorption data at 24 C were obtained for Empro 80, a Dairy Products Laboratory experimental high-protein whey powder, and for lyophilized preparations of its high and low molecular weight fractions obtained by dialysis. In addition, less detailed sorption data were obtained for three whey powders by the weighing bottle-desiccator method. Samples of powders contained 73, 83, and 87% protein were placed in weighing bottles and equilibrated against humid atmospheres controlled with saturated salt solutions (15). These measurements were at 24 and 35 C.

Standard methods were used for chemical analysis of the whey powders. Lactose was determined by the procedure of Fox et al. (8), and protein was calculated from nitrogen content from the standard micro-Kjeldahl procedure (1). The denatured or insoluble protein content of the powders was determined by the centrifugation method of Guy et al. (9).

Results and Discussion

Data in Table 1 show that products containing more low molecular weight materials, i.e. lactose and salts, bind more water. Measurements with fractionated samples (Table 2) clearly demonstrated that the dialyzable fraction bound at least twice as much water as the protein fraction. When these values for the dialyzable fraction are correlated with the chemical data in Table 1, it is apparent that the low molecular weight components other than lactose are more responsible for water binding. This agrees with data reported for H_2O vapor sorption by lactose and a synthetic milk salt mixture (3). Further research is necessary to determine which ionic species, contributing to the ash, are most significant in water binding in these whey protein concentrates.

Water vapor sorption data at 24 C for powders containing 73, 83, and 87% whey protein

TABLE 2. Bound water in dispersed fractions of whey protein concentrates.

Protein concentrate	grams H_2O bound / gram dry protein	grams H_2O bound / gram dry dialysate
A	.49	1.17
B	.52	1.86
F	.45	1.17

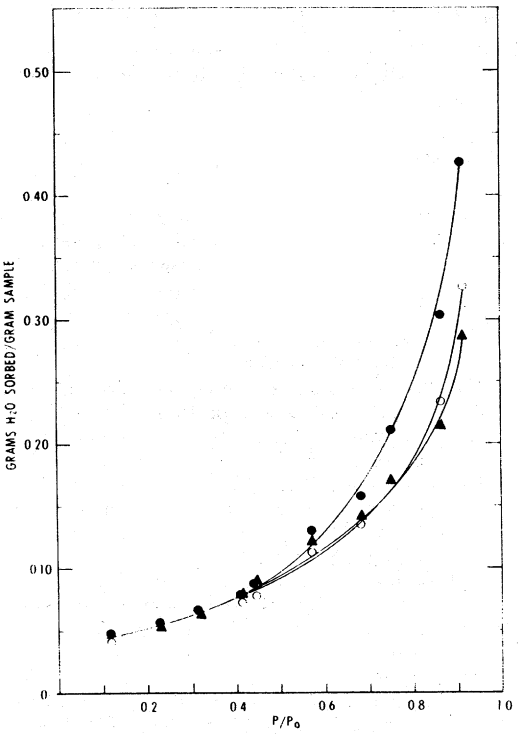

FIG. 1. Water vapor sorption on dehydrated whey protein concentrates at 24 C. ● — D; ○ — E; ▲ — G (see Table 1).

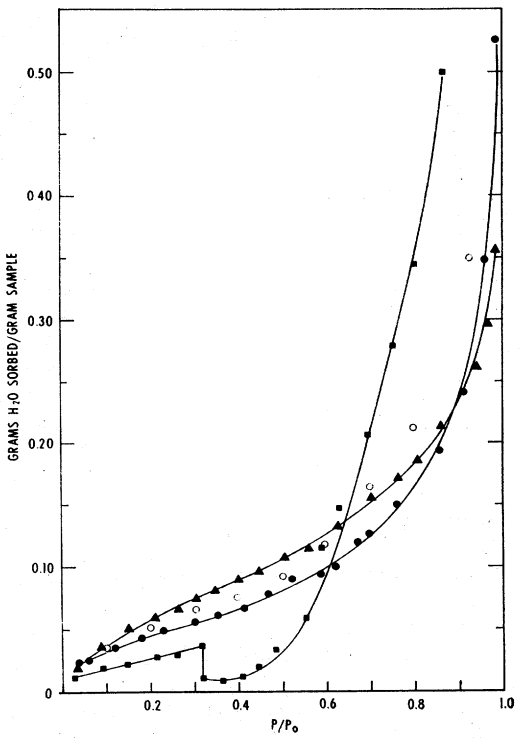

FIG. 2. Water vapor sorption at 24 C on dehydrated whey protein concentrate (Empro 80) and dialyzed fractions thereof. ● — Empro 80; ▲ — nondialyzable fraction; ■ — dialysate; ○ — Empro 80, calculated from fractions.

are presented graphically in the isotherms of Fig. 1. Identical sorption properties are displayed by all three powders at lower water vapor pressures, but above $.4P_0$ the powders containing more lactose and salts sorbed more water. All three isotherms are smooth and do not exhibit the discontinuities encountered with whey powders (2) since very little amorphous lactose is in these powders. Measurements at 24 and 35 C showed an inverse temperature dependence for water sorption by the whey protein concentrates. This is normal for physical adsorption but differs from that with milk or whey powders containing large amounts of amorphous lactose (4) because in those instances the sorbed water is more mobile prior to crystallization of lactose.

The sorption data for protein powder Empro-80 and its fractions (Fig. 2) show the changing roles of these fractions in sorption as a function of relative pressure. Agreement is close between the observed isotherm and that calculated from the sorption data for the fractions indicating the additive nature of water sorption in this system.

Calculated bound water values from the fractional data in Table 2 are in almost exact agreement with the experimental bound water values for the same concentrates (Table 1). For example, a calculated value of 1.09 g water bound/g solids is in excellent agreement with the experimental value of 1.11 g water/g solids obtained with protein concentrate B. After recombining the fractions of this powder, a bound water value of 1.06 g H_2O/g solids was obtained, which agrees with the previous values.

Labuza (12) has suggested that water sorption in foods is additive, but he pointed out the need for research to determine the water binding of the components in complex food mixtures. Actually, Briggs (7) postulated this general concept of the additivity of water binding by nonreacted components of mixed systems 40 yr ago in a theoretical treatment of bound water in colloids. This theory was related to earlier experimental work (6) including water binding to sodium and calcium caseinates. Briggs' principle, although stated in general terms, may not operate in all cases, as Redfern

and Patrick observed with silica gel (14). Each mixed system of interest must be studied experimentally as suggested by Labuza (12). We have demonstrated the validity of this concept experimentally in the present work and in earlier studies with milk powders (3).

The results of this study demonstrate the additivity of water binding for concentrated aqueous dispersions containing as much as 75% water as well as for water vapor-solid interactions. Considering the gravimetric and calorimetric data together provides insight into the relative importance of the high and low molecular weight fractions in water binding. Below $P/P_0 \approx .7$ on the adsorption isotherm (Fig. 2) water is sorbed primarily by the macromolecular component; however, at higher relative pressures the dialyzable fraction sorbs far more water forming a concentrated aqueous solution. As the saturation pressure is approached in these systems, the mass of water vapor sorbed begins to equal the amount bound in the corresponding aqueous dispersion.

Present (Table 2) and earlier (5) findings that there are .5 g of unfreezable water per g of protein in pure protein solutions agree reasonably with the data of Hasl and Pauly (10), who reported .3 g H_2O/g dry bovine serum albumin (BSA) as the caloric bound water and .54 g H_20/g dry BSA as the total water atmosphere around each molecule of bovine serum albumin.

Consideration of the percentage of insoluble protein (Table 1) in these concentrates suggests that thermal denaturation has little effect, if any, on unfreezable water or sorbed water. Values of .47, .56, .45, and .49 g of bound water per g of insoluble protein pellets from concentrates A, B, D, and F agree with those for the total protein fraction of the same concentrate. Bound water values were almost identical before and after heating (1 h, 85 C) wet samples of pure whey protein and concentrates D and F in the calorimeter. The loss in protein solubility through thermal denaturation has little or no effect on the capacity to bind water against freezing or to adsorb water from the vapor phase.

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